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## Supplemental information

### *In vivo* targeting of a variant causing vanishing white matter using CRISPR/Cas9

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## Supplementary information

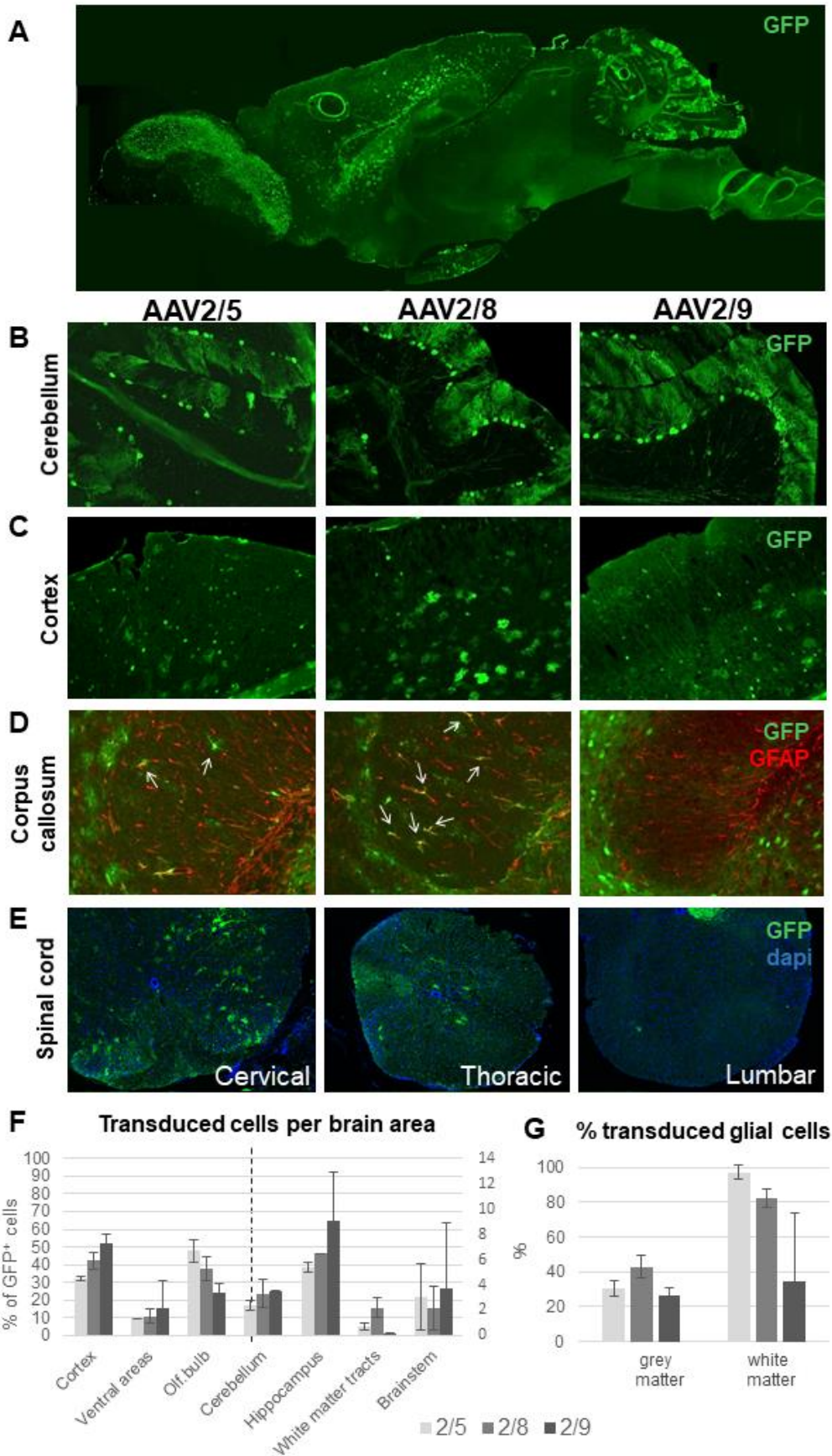
### Materials & methods

**Table S1. Number of animals used per assay and statistical descriptors**

Assay	N	M + SD (or median + range)	95% confidence interval & effect size
Serotype pilot spread  Total n=7 If n=1, 2 slices were analyzed per animal	<b>AAV2/5:</b> 5 weeks n=1 + 2 months n=1	Cortex: M=32.3, SD=1.39 Ventral areas: M=9.7, SD=0.07 Olf. bulb: M=47.7, SD=6.32 Cerebellum: M=2.4, SD=0.39 Hippocampus: M=5.4, SD=0.37 White matter: M=0.7, SD=0.31 Brain stem: M=3.0, SD=2.58	N/A
	<b>AAV2/8:</b> 5 weeks n=2 + 2 months n=1	Cortex: M=42.2, SD=4.65 Ventral areas: M=11.0, SD=3.68 Olf. bulb: M=37.6, SD=6.80 Cerebellum: M=3.3, SD=1.15 Hippocampus: M=6.5, SD=N/A White matter: M=2.2, SD=0.78 Brain stem: M=2.1, SD=1.69	
	<b>AAV2/9:</b> 5 weeks n=1 + 2 months n=1	Cortex: M=51.8, SD=5.49 Ventral areas: M=15.5, SD=15.21 Olf. bulb: M=24.3, SD=4.85 Cerebellum: M=3.5, SD=0.13 Hippocampus: M=9.1, SD=3.89 White matter: M=0.1, SD=0.14 Brain stem: M=7.4, SD=N/A	
Serotype pilot astrocytic tropism  Total n=7 If n=1, 2 slices were analyzed per animal	<b>AAV2/5:</b> 5 weeks n=1 + 2 months n=1	Grey matter: M=30.8, SD=4.47 White matter: M=97.2, SD=3.93	N/A
	<b>AAV2/8:</b> 5 weeks n=2 + 2 months n=1	Grey matter: M=42.8, SD=6.39 White matter: M=82.4, SD=5.18	
	<b>AAV2/9:</b> 5 weeks n=1 + 2 months n=1	Grey matter: M=26.4, SD=4.23 White matter: M=34.3, SD=39.83	
Balance beam latency  Total n=79	<b>CRISPR<sup>hom</sup>: n=10</b> <b>CRISPR<sup>het</sup>: n=24</b> <b>Control<sup>hom</sup>: n=21</b> <b>Control<sup>het</sup>: n=24</b>	M=23.2, SD=9.29 M=12.3, SD=6.41 M=18.3, SD=8.08 M=4.5, SD=1.45	16.60-29,88 9.55-24.97 14.59+21.95 3.84+5.06 $\eta^2 = .525$
Balance beam accuracy  Total n=79	<b>CRISPR<sup>hom</sup>: n=10</b> <b>CRISPR<sup>het</sup>: n=24</b> <b>Control<sup>hom</sup>: n=21</b> <b>Control<sup>het</sup>: n=24</b>	M=11.9, SD=4.81 M=4.9, SD=3.41 M=18.3, SD=8.08 M=4.5, SD=1.45	8.42-15.30 3.43-6.31 14.59-21.95

			3.84-5.06 $\eta^2 = .690$
Grip strength Total n=80	<b>CRISPR<sup>hom</sup>: n=10</b> <b>CRISPR<sup>het</sup>: n=24</b> <b>Control<sup>hom</sup>: n=21</b> <b>Control<sup>het</sup>: n=25</b>	M=1.4, SD=0.23 M=1.7, SD=0.22 M=1.6, SD=0.19 M=1.9, SD=0.18	1.28-1.61 1.63-1.81 1.54-1.71 1.87-2.02 $\eta^2 = .422$
Next Generation Sequencing- NHEJ Total n=97	<b>CRISPR: n=51</b> <b>CRISPR<sup>hom</sup>: n=21</b> <b>CRISPR<sup>het</sup>: n=30</b> <b>Control: n=46</b> <b>Control<sup>hom</sup>: n=23</b> <b>Control<sup>het</sup>: n=23</b>	M=4.6, SD=1.72 M=4.9, SD=1.92 M=4.5, SD=1.55 M=0.08, SD=0.05 M=0.05, SD=0.05 M=0.08, SD=0.05	4.16-5.12 4.01-5.76 3.89-5.05 0.07-0.10 0.07-0.10 0.06-0.10 CRISPR vs. controls: Cohens's d =3.792
Next Generation Sequencing- HDR Total n=97	<b>CRISPR: n=51</b> <b>(CRISPR<sup>hom</sup>: n=21</b> <b>CRISPR<sup>het</sup>: n=30)</b> <b>Control: n=46</b> <b>(Control<sup>hom</sup>: n=23</b> <b>Control<sup>het</sup>: n=23)</b>	M=0.3, SD= 0.17 M=0.3, SD=0.20 M=0.2, SD=0.13 M=0.004, SD=0.01 M=0.1, SD=0.05 M=0.08, SD=0.05	0.22-0.31 0.24-0.42 0.17-0.27 0.001-0.006 0.07-0.10 0.06-0.10 CRISPR vs. controls: $\eta^2 = .778$
Weight at 42 days old Total n=149	<b>CRISPR<sup>hom</sup>: n=30</b> <b>CRISPR<sup>het</sup>: n=42</b> <b>Control<sup>hom</sup>: n=40</b> <b>Control<sup>het</sup>: n=37</b>	M=13.7, SD=2.45 M=16.4, SD=3.31 M=15.4, SD=2.50 M=15.6, SD=2.48	12.30-15.00 15.26-17.57 14.50-16.33 14.75-16.43 CRISPR <sup>hom</sup> vs. CRISPR <sup>het</sup> : $\eta^2 = .852$
Weight at 7 months old Total n=80	<b>CRISPR<sup>hom</sup>: n=10</b> <b>CRISPR<sup>het</sup>: n=24</b> <b>Control<sup>hom</sup>: n=21</b> <b>Control<sup>het</sup>: n=25</b>	M=19.6, SD=2.80 M=28.0, SD=7.08 M=23.6, SD=3.90 M=32.5, SD=5.11	17.60-21.60 24.98-30.96 21.87-25.42 30.35-24.56 $\eta^2 = .482$
Neuroscore at 7 months old Total n=80	<b>CRISPR<sup>hom</sup>: n=10</b> <b>CRISPR<sup>het</sup>: n=24</b> <b>Control<sup>hom</sup>: n=21</b> <b>Control<sup>het</sup>: n=25</b>	M=0.60, SD=0.46 M=0.63, SD=0.58 M=0.48, SD=0.33 M=0.06, SD=0.22	0.27-0.93 0.38-0.87 0.32-0.63 -0.03-0.15 $\eta^2 = .264$
H&E Total n=10	<b>CRISPR<sup>hom</sup>:</b> 34-44 days old n=4 9 months old n=1 <b>CRISPR<sup>het</sup>:</b> 34-44 days old n=1 9 months old n=1 <b>Control<sup>hom</sup>:</b> 9 months old n=2 <b>Control<sup>het</sup>:</b> 9 months old n=1	N/A	N/A

Timed culling of animals	12 weeks old <b>Control<sup>hom</sup></b> n=4 <b>Control<sup>het</sup></b> n=4
	5 months old <b>CRISPR<sup>het</sup></b> n= 4 <b>Control<sup>hom</sup></b> n= 15 <b>Control<sup>het</sup></b> n=8
	8 months old <b>CRISPR<sup>het</sup></b> n=12 <b>Control<sup>hom</sup></b> n=9 <b>Control<sup>het</sup></b> n=12
	9 months old <b>CRISPR<sup>hom</sup></b> n=10 <b>CRISPR<sup>het</sup></b> n=13 <b>Control<sup>hom</sup></b> n=12 <b>Control<sup>het</sup></b> n= 13



**Supplemental Figure 1. AAV serotypes were tested for astrocytic transduction *in vivo*.** (A) Neonatal mice received intracerebroventricular injections of AAV, which

resulted in a wide spread of the virus at 5 weeks of age. Expression of CMV-GFP (green), conferred by AAV with chimeric serotypes AAV2/5, AAV2/8, or AAV2/9, was seen in the **(B)** cerebellum and **(C)** cortex. **(D)** In the corpus callosum at 5 weeks of age, co-localization of GFP<sup>+</sup> cells with GFAP (red) is indicated by arrows. **(E)** The spinal cord showed transduced cells at the cervical, thoracic and lumbar portions at 9 months post-injection of AAV2/8. **(F)** The number of transduced cells per region are quantified (dashed line assigns regions to the two different y-axes), and **(G)** expressed in the percentage of glial cells of the total number of transduced cells. n=2 animals for AAV2/5 and AAV2/9, and n=3 animals for AAV2/8 until 5 or 8 weeks of age. n=24 for AAV2/8 until 9 months of age.